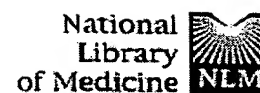


Exhibit 10



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1: Transgenic Res. 1995 Mar;4(2):87-104.

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Expression of bacterial cysteine biosynthesis genes in transgenic mice and sheep: toward a new in vivo amino acid biosynthesis pathway and improved wool growth.

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It is possible to improve wool growth through increasing the supply of cysteine available for protein synthesis and cell division in the wool follicle. As mice can only synthesise cysteine indirectly from methionine via trans-sulphur expression of transgenes encoding microbial cysteine biosynthesis enzymes, we provide a more efficient pathway to cysteine synthesis in the sheep. If expression in the rumen epithelium, the abundant sulphide, produced by ruminal microorganisms and normally excreted, could be captured for conversion to cysteine. This paper describes the characterisation of expression of the cysteine biosynthesis genes of *Salmonella typhimurium*, *cysE*, *cysM* and *cysK*, and *cysEM*, *cysME* and *cysKE* genes as transgenes in mice and sheep. The libraries of transgenes were constructed with each gene driven by a separate promoter with the Rous sarcoma virus long terminal repeat (RSVLTR) promoter or mouse phosphoglycerate kinase-1 (mPgk-1) promoter, and with human growth hormone (hGH) polyadenylation sequences. Transgenesis of mice with the RSVLTR-*cysE* gene afforded tissue-specific, heritable expression of the cysteine. Despite high levels of expression in a number of tissues, extremely low levels of expression occurred in the stomach and small intestine. Results of a concurrent sheep transgenesis experiment using the RSVLTR-*cysEM* and -*cysME* libraries revealed that the RSVLTR promoter was inadequate for expression in the rumen. Moreover, instability of transgenes containing the RSVLTR sequence was observed. Expression of mPgk-*cysME* and -*cysKE* linked transgenes in tissues of the mice examined, including the stomach and small intestine, suggested this promoter to be a better candidate for expression of these transgenes in analogous tissues of sheep. However, a subsequent sheep transgenesis experiment indicated that use of the mPgk-1 promoter, active ubiquitously and early in development, may be inappropriate for expression of the cysteine biosynthesis transgenes. In summary, these results indicate that enzymically active bacterial cysteine biosynthesis gene products can be coexpressed in mammalian cells in vivo but that expression of the genes should be spatio-temporally restricted to adult sheep rumen epithelium.

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